Technische Universiteit
Eindhoven
University of Technology

iGEM TU/e 2014
Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2014.igem.org/Team:TU_Eindhoven

Date
11 August 2014

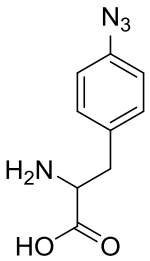
Protein Expression Curve

Technische Universiteit
Eindhoven
University of Technology

Table of contents

Title
Protein Expression Curve

1 Stock solutions



Molecular Weight: 206.20
pAzF

- 10 mM pAzF in 2YT with 5 v% DMSO

2 Growth of small bacterial cultures

- Fill 15 mL sterile culture tubes with 5 mL 2YT. Work near the Bunsen burner flame
- Add 5 µL kanamycin (30 µg/mL) and 5 µL chloramphenicol (25 µg/mL) to the cultures
- Pick cells from the glycerol stock using a sterile pipette tip and throw the pipette tip in the culture tube
- Grow the bacteria overnight at 37°C and 250 rpm

3 Protein expression

- Transfer 4 mL of the small culture to new 120 mL 2YT culture (with 100 µL kanamycin (30 µg/mL) and 100 µL chloramphenicol (25 µg/mL))
- Grow the bacteria at 37 °C and 250 rpm until OD=0.6 (measure blank with 2YT)
- When OD = 0.6 wrap culture tube in aluminum foil and continue with next step
- Divide the 120 mL culture into two cultures of 50 mL
- For a 50 mL culture: add 56.2 µL IPTG from the 1M stock, 562.4 µL of arabinose from the 20% stock and 5624.3 µL of unnatural amino acid from the 10 mM stock to the culture wrapped in aluminum foil, in order to get end concentrations of 1 mM, 0.2% and 1 mM, respectively
- Put one 50 mL culture in the fridge and continue with the other 50 mL culture
- Take a 5 mL sample each hour for five hours according to the scheme below

---

- Spin down the cells for 15 min at 3,000 xg.
- Discard supernatant.
- Add 1 ml PBS and transfer to 1,5 mL Eppendorf tube.
- Spin down the cells for 1 min at 13,400 rpm.
- Discard supernatant
- Add 1 ml PBS
- Fill 1 OD cuvette with 950 µL dH2O and 50 µL PBS for blank OD measurement.
- Fill 1 OD cuvette with 950 µL dH2O and 50 µL of culture sample (dilution of 20x)
- Measure OD, this has to be lower than 1, otherwise make a higher dilution.
- Calculate OD of culture sample. (If you made a dilution of 20X then the OD of the culture is 20X the OD of the dilution.

- Calculate amount of cells in culture.
  http://www.genomics.agilent.com/biocalculators/calcODBacterial.jsp?_requestid=826255
  Your answer of the previous step is in bacteria/mL.
- Calculate amount of PBS you have to add to obtain a final concentration of 109 cells/mL. Use the following formula:

This will give you the number you have to dilute your solution:

$$Dilution = \frac{\left[\frac{Bacteria}{mL}\right]}{10^9}.$$

Amount of cells:

$$Amount\ of\ cells\ (mL) = 0.2/Dilution$$

because you will make 0.2 mL of 10^9 cells

Amount of PBS-BSA 0,1%:

$$Amount\ of\ PBS\ (mL) = 0.2 - Amount\ of\ cells$$

will make 0.2 mL of 10^9 cells

- Add 1.21 µL DBCO-5/6-TAMRA (5 mM) to 200 µL of each sample
- React for 1h in shaking block at 4°C
- In the meanwhile, set the incubator so that shaking and temperature increase starts at the according time at night
- Spin down the cells for 10 min at 13,400 rpm
- In the meanwhile, prepare a ‘nulmeting’ by adding 1.21 µL DBCO-5/6-TAMRA (5 mM) to 200 µL PBS
- Transfer supernatant into a new Eppendorf tube
- Perform Nanodrop for each sample of supernatant and the ‘nulmeting’

- The next day: repeat the protocol from the black line